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MECHANISMS ACCELERATING MUSCLE ATROPHY IN CATABOLIC DISEASES

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INTRODUCTION

Loss of lean body mass, predominantly muscle, is a serious complication for patients afflicted with virtually any disease because protein malnutrition in hospitalized patients is associated with excessive morbidity and mortality (1). The potential causes of protein malnutrition include an inadequate diet but the mechanism(s) leading to loss of muscle mass in such patients is not so simple since feeding more calories and protein to catabolic patients does not result in positive nitrogen balance nor an increase in muscle mass (2). Instead, the cause of muscle loss is an imbalance in protein turnover because of accelerated protein degradation and/or suppressed protein synthesis. Since the daily rate of protein turnover exceeds protein intake by more than 3-fold when dietary protein is above the recommended daily allowance of protein, even a small change in protein turnover will upset protein balance and if the change is persistent, there will be a major loss of protein stores (Figure 1). In fact, the major cause of the decline in muscle mass that occurs in catabolic illnesses is an acceleration of protein degradation. Recent discoveries using animal models of catabolic illnesses have provided insights into the mechanisms stimulating muscle protein degradation: in all the conditions studied to date, common mechanisms when muscles atrophy (3).

PHYSIOLOGIC IMPORTANCE OF PROTEIN DEGRADATION

The information depicted in Figure 1 shows that the rates of protein degradation must be highly regulated to match changes in the rates of protein synthesis precisely in widely varied proteins (in terms of half-lives) in order to maintain protein balance. In some ways, the daily loss of so much protein in processes of protein degradation seems counterproductive but in fact, the continual destruction of cellular proteins serves several important homeostatic

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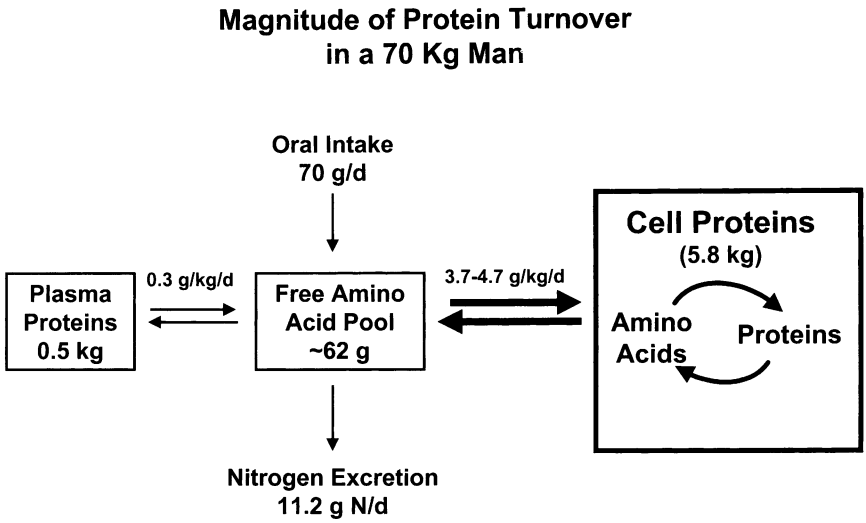

FIG. 1. The daily flux of protein in a 70 kg adult in nitrogen balance while eating 1 g protein/kg/day and excreting the equivalent amount of protein as nitrogen. The turnover of cellular proteins is substantially larger than the intake of protein and exceeds the turnover of plasma proteins by 10-fold.

functions. Firstly, the rapid removal of critical regulatory proteins (e.g., transcription factors or enzymes and inhibitory factors) is essential for regulating the growth of cells and metabolic processes. Secondly, the rapid degradation of specific proteins permits adaptations to any physiologic condition that produces a change in cell composition. For example, hepatic enzymes catalyzing glucose storage disappear and the synthesis of gluconeogenic enzymes increases within hours of beginning a fast and these changes rapidly reverse after feeding. Thirdly, the degradation of cellular proteins serves an essential control mechanism that selectively eliminates abnormally folded or damaged proteins that have arisen by mutations, biosynthetic errors, or through damage by oxidation or denaturation. For example, globins that are folded abnormally in patients with hemoglobinopathies are degraded within minutes after synthesis. Fourthly, in producing antigens, protein degradation plays a critical role in the normal functioning of the immune system. Finally, when calories are inadequate or there is a catabolic disease, the overall breakdown of cell proteins (especially those in muscle) increases to provide the organism with amino acids that are essential for gluconeogenesis and the synthesis of new proteins (4).

CELLULAR PATHWAYS OF PROTEIN DEGRADATION

Early work established that lysosomes perform an important function by degrading proteins as well as large glycolipids and constituents of the cell membrane and it was suspected that lysosomes were primarily responsible for the continual turnover of cellular proteins. It is now established, however, that protein degradation also occurs in the cytoplasm, endoplasmic reticulum, the nucleus and in mitochondria (3). On the other hand, the mechanisms by which cellular proteins destined for degradation are recognized and how specific proteolytic pathways become activated are still not well understood.

Regarding the pathways in which specific proteins are degraded (Figure 2), extracellular proteins (e.g., plasma proteins, hormones, phagocytosed bacteria, etc.) are engulfed by endocytosis and degraded within lysosomes (the intracellular organelles that contain proteases with an acidic optimum pH such as Cathepsin B, H and D). In addition, lysosomes degrade foreign proteins to generate peptides that are presented to the immune system in association with MHC Class II molecules. Finally, cytosolic proteins taken up by autophagic vacuoles are degraded in lysosomes. In the liver and possibly other organs, this process is accelerated when levels of amino acids and insulin are low.

Other cytosolic proteolytic systems include the calpains which are calcium-activated, cysteine proteases and the ATP-dependent and

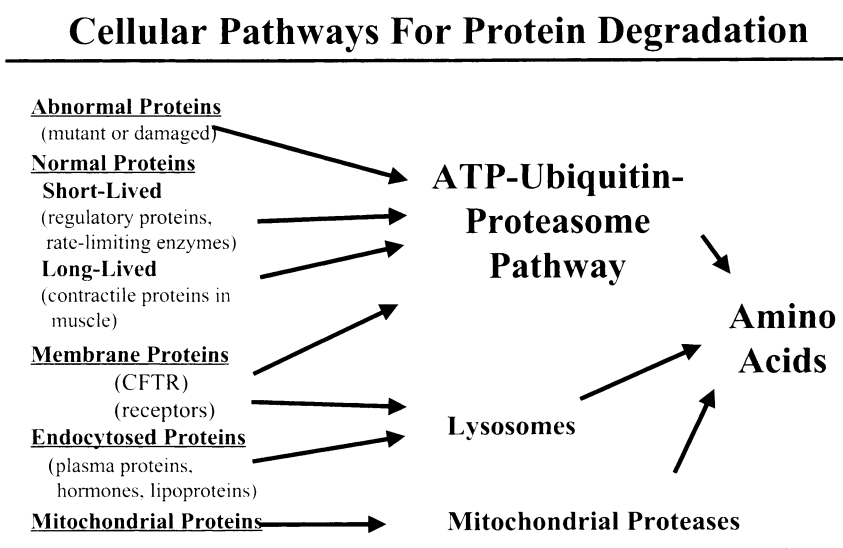

FIG. 2. Specific types of cellular proteins are degraded in the different pathways of cellular proteolysis. The major system degrading proteins is the ATP-ubiquitin-proteasome pathway.

ATP-independent proteolytic pathways. The calpains appear to be activated when cells are injured, leading to a rise in cytosolic calcium, but the function of calpains in the normal turnover of cellular proteins is unclear (5). There are reports that calpains do participate in the initial disruption of the complex secondary structure of muscle (6,7).

It is now clear that the bulk of all cellular proteins is degraded by the ATP-ubiquitin-proteasome dependent pathway (Ub-P' some system) (8,9). Initially, this pathway was shown to be responsible for the degradation of abnormal (e.g., misfolded) proteins and short-lived proteins involved in regulating cellular processes but more recent reports indicate that this system is also responsible for the turnover of long-lived proteins in cells (3,4). This system also generates peptides presented on MHC Class I molecules during the immune response (8).

Finally, there is the ATP-independent proteolytic pathway that can degrade cellular proteins. Substrates degraded by this poorly-understood pathway are not well characterized and its importance in the control of protein balance in cells has not been identified.

The contribution of each of these pathways to the control of the mass of protein in muscle has been estimated in skeletal muscle using inhibitors of lysosomes, of calcium-activated proteases and the impact of ATP depletion and, more recently, inhibitors of the proteasome (9–13). These studies were carried out in muscles that had been isolated from rats after establishing a catabolic condition that mimics a human disease. The results demonstrate that the Ub-P' some pathway is almost uniformly stimulated by catabolic conditions (Table 1).

TABLE 1
Muscle Protein Turnover and the ATP-Ubiquitin-Proteasome Pathway

Experimental Model	Human Disease
fasting	eating disorders
metabolic acidosis	renal tubular defects, diabetes
kidney failure	acute and chronic uremia
muscle denervation	neuromuscular disease, immobilization
thermal injury	burns
endotoxins	sepsis, AIDS
tumor implantation	cancer cachexia
glucocorticoids	Cushing's Disease
thyroid hormone	Graves' Disease
insulinopenia	diabetes

Studies of experimental models of human diseases reveal that loss of muscle mass involves a common pathway, the ubiquitin-proteasome system. In all of these models, there is evidence of increased degradation of muscle proteins in the ubiquitin-proteasome pathway and increased levels of mRNAs encoding components of the pathway.

Proteins destined for hydrolysis by the Ub-P' some system are modified by covalent binding of ubiquitin molecules, marking them as target proteins for rapid, ATP-dependent degradation by the 26S proteasome complex (3,14,15). The initial step in the Ub-P' some pathway (Figure 3) is formation by the ubiquitin-activating enzyme of a high energy, ubiquitin-thioester intermediate. The activated ubiquitin is then transferred to one of many ubiquitin-carrier proteins (E2's) before it is linked to the ϵ-amino of a lysine in the protein substrate. The latter reaction is catalyzed by a ubiquitin-protein ligase, E3. In successive reactions, a chain of 5 or more ubiquitin molecules is formed, serving as a marker recognized by the 26S proteasome. The 26S proteasome consists of four rings composed of distinct subunits (the 20S proteasome) and 19S cap particles that are on both ends of the cylindrical structure. These caps contain molecules that recognize the ubiquitin-protein conjugate as well as ATPases that presumably function to unfold the protein substrate. The active sites of the 26S proteasome are contained on the inner surface of a cylindrical core made up by the four rings (15). Thus, there are several steps which could be

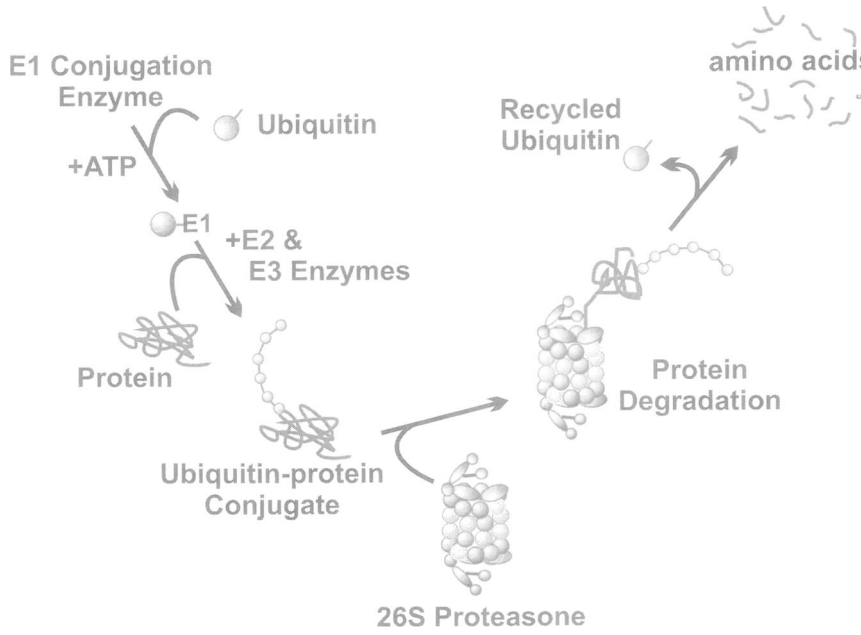

FIG. 3. A schematic depiction of the multiple steps involved in degradation of a protein in the ubiquitin-proteasome system. This complex pathway has several sites where the rate of protein degradation could be regulated and this is necessary to prevent excessive protein loss.

stimulated or suppressed to regulate the process of protein degradation by the Ub-P' some system.

MUSCLE PROTEIN DEGRADATION IN CATABOLIC CONDITIONS

Early measurements of protein turnover established that nitrogen balance as an index of the loss of muscle mass is substantial in patients with sepsis, burns or after injury (Table 2). The next level of understanding came when it was shown that accelerated protein degradation is responsible for the muscle protein loss that occurs in animal models of these diseases. For example, we have been interested in the mechanisms causing loss of muscle protein in kidney disease because malnutrition and muscle atrophy are common in dialysis patients (16). In searching for the factors that stimulate muscle protein catabolism, we evaluated the impact of metabolic acidosis because it is common in patients with kidney disease and it can be experimentally manipulated by feeding $NaHCO_3$. In the initial series of investigations we found that protein degradation is accelerated in isolated muscles of rats that had been fed NH_4Cl to induce metabolic acidosis; this response was shown to require glucocorticoids (3). In studies of intact rats, we then confirmed that glucocorticoids are required for the stimulation of muscle protein breakdown and the increased levels of mRNAs encoding components of the Ub-P' some system (17,18). These studies were extended in rats to show that the metabolic acidosis caused by chronic renal failure (CRF) stimulates protein breakdown in muscle (19) and later, that the proteolytic pathway stimulated by metabolic acidosis is the Ub-P' some pathway (11). In the evaluation of CRF, we used an inhibitor of the proteasome and proved that activation of the Ub-P' some pathway is responsible for the accelerated muscle proteolysis (9). An interesting feature of all disorders that have been shown to activate

TABLE 2
Magnitude of nitrogen losses in catabolic illnesses

Illness	Cumulative losses in 7 days	
	Nitrogen (g)	Protein equivalent (kg)
Extensive burn	119	0.71
Multiple injuries	105	0.70
Long-bone fracture	81	0.50
Major surgery	35	0.22
Acute renal failure (toxin)	16.5–24.5	0.11–0.15
Minor surgery	16.4	0.11
Pneumonia	41	0.31

the Ub-P' some system is the associated increase in the levels of mRNAs encoding ubiquitin and subunits of the proteasome; the same occurs with metabolic acidosis (3,11).

Several groups of investigators have reported that metabolic acidosis stimulates protein degradation in patients. Both normal adults and predialysis CRF patients respond to metabolic acidosis by increasing protein degradation (20,21). Moreover, the accelerated protein breakdown occurring in CAPD or hemodialysis patients who have acidosis is substantially reduced by giving them $NaHCO_3$ to correct metabolic acidosis (22,23). Furthermore, hypoalbuminemia (the primary indicator of mortality in dialysis patients (24)) can be significantly improved by correcting metabolic acidosis (25). Perhaps the most convincing evidence that acidosis stimulates protein catabolism in uremic patients is found in the report of Stein et al (26). They carried out a year-long, randomized trial of the influence of correcting acidosis on the nutritional status of CAPD patients and reported a significant increase in both weight and muscle mass when acidosis was corrected. We are collaborating with this group to carry out a similar CAPD protocol and we find that correction of acidosis is associated with a significant decrease in the level of ubiquitin mRNA measured in muscle. Others report that the levels of mRNA encoding ubiquitin and subunits of the proteasome are increased in muscle of patients with different catabolic disorders (27,28). These results provide evidence for activation of the Ub-P' some pathway in patients, as suggested by results from experimental models of catabolic illnesses.

SIGNALS ACTIVATING THE PROTEOLYSIS IN UREMIA

Even though there is overwhelming evidence that the metabolic acidosis of CRF causes muscle atrophy in rats by accelerating protein breakdown, identifying the signals activating protein catabolism has been difficult. Potential signals include a low cell pH, glucocorticoids, impaired insulin responses and cytokines. For example, in cultured muscle cells, the intracellular pH decreases when the media is acidified and protein degradation rises (29,30). However, the muscle cell pH (measured by NMR) in anesthetized, normal rats with metabolic acidosis, reveals only a small decrease, and in CRF-rats with acidosis, there is no decrease in muscle pH nor any abnormality in the recovery of pH following intracellular acidification. The latter was especially surprising considering our demonstration that ion transporters in muscle from rats with CRF function abnormally (31–34). These results make it highly unlikely that a

decrease in intracellular pH in CRF is the principal cause that activates the Ub-P' some system in muscle. It is still possible that metabolic acidosis acts indirectly to stimulate cellular catabolism. For example, in isolated monocytes, acidification stimulates tumor necrosis factor (TNFα) production while in muscle, metabolic acidosis causes insulin resistance (35,36) and both of these responses could stimulate catabolic mechanisms.

Glucocorticoids are a second candidate for the signal that activates muscle protein degradation because glucocorticoid production is increased in uremia (19) and we found that glucocorticoids are necessary for the accelerated muscle protein breakdown stimulated by metabolic acidosis (17,37). The important point is that physiologic levels of glucocorticoids alone do not activate the system, indicating that glucocorticoids must be acting in a permissive, rather than a primary role. In fact, it has been shown that glucocorticoids are required for activation of the Ub-P' some pathway and muscle protein breakdown in rats with acidosis, starvation, sepsis and acute diabetes (17,37–40).

A third potential signal is depressed responsiveness to insulin because a reduced insulin level or impaired responses to insulin is common to several catabolic conditions (e.g., uremia, sepsis, starvation). To identify how a low insulin level or impaired response to insulin might influence the Ub-P' some system, we studied streptozotocin-treated rats (12). There was accelerated muscle protein degradation which was blocked by a proteasome inhibitor proving that the Ub-P' some pathway is responsible for the muscle atrophy that occurs with uncontrolled diabetes mellitus. There also were high levels of mRNAs encoding ubiquitin, ubiquitin-conjugating enzyme ($E2_{14k}$) and proteasome subunits due to increased transcription of these genes (40) and increased rates of conjugation of ubiquitin to the proteins in muscle (41). All of these responses were independent of the metabolic acidosis of diabetes but as with acidosis and starvation, were dependent on glucocorticoids (40).

Finally, cytokines could be a factor that activates the Ub-P' some pathway (42). Firstly, the system is activated in muscle of rats with burns, cancer, or sepsis and each of these conditions is associated with high circulating levels of cytokines (3). Secondly, cytokines could even be the signal in uremia because surveys of undernourished hemodialysis patients show that these patients have high serum markers of inflammation such as C-reactive protein and α_2-macroglobulin (43). Thirdly, evaluation of the responses to an infusion of cytokines revealed that TNFα stimulates protein degradation in rats (44,45). So

far, a mechanism by which a specific cytokine activates the Ub-P' some pathway has not been identified.

It is also possible that several signals act independently or in combination to stimulate protein degradation in muscle. We tested for interactions between glucocorticoids and cytokines by studying how these factors act to regulate transcription of genes encoding components of the ubiquitin-proteasome pathway in L6 muscle cells. The C3 proteasome subunit gene was studied because transcription of this gene is increased when the ubiquitin-proteasome system is activated. Our preliminary results indicate that NF-κB suppresses C3 subunit transcription but this effect is blocked if glucocorticoids are present (46). These results may provide an explanation for the permissive influence of glucocorticoids in raising mRNAs of the ubiquitin-proteasome system and they point out that multiple TNFα-signaling pathways could be involved in regulating proteolysis.

SUMMARY

In summary, muscle protein loss in uremia is related to activation of the ubiquitin-proteasome proteolytic system to degrade muscle proteins. This response invariably includes increased transcription of genes encoding components of this pathway, suggesting that these illnesses stimulate a program of catabolism. Signals that could activate muscle protein degradation by this system in CRF include metabolic acidosis, impaired response to insulin and high circulating levels of cytokines. The activation mechanism also involves glucocorticoids which are necessary but not sufficient to activate protein degradation in muscle.

REFERENCES

1. Ziegler TR, Gatzen C, Wilmore DW. Strategies for attenuating protein-catabolic responses in the critically ill. Annu. Rev. Med. 1994;45:459–80.
2. Shaw JHF, Wildbore M, Wolfe RR. Whole body protein kinetics in severely septic patients. Ann. Surg. 1987;205:288–94.
3. Mitch WE, Goldberg AL. Mechanisms of muscle wasting: The role of the ubiquitin-proteasome system. N. Engl. J. Med. 1996;335:1897–905.
4. Lecker SH, Solomon V, Mitch WE, Goldberg AL. Muscle protein breakdown and the critical role of the ubiquitin-proteasome pathway in normal and disease states. J. Nutr. 1999;129:227S–37S.
5. Tan FC, Goll DE, Otsuka Y. Some properties of the millimolar Ca2+-dependent proteinase from bovine cardiac muscle. J. Mol. Cell. Cardiol. 1988;20:983–97.
6. Huang J, Forsberg NE. Role of calpain in skeletal-muscle protein degradation. Proc. Natl. Acad. Sci. USA 1998;95:12100–5.
7. Combaret L, Ralliere C, Taillandier D, Tanaka K, Attaix D. Manipulation of the

ubiquitin-proteasome pathway in cachexia: pentoxifylline suppresses the activation of 20S and 26S proteasomes in muscles from tumor-bearing rats. Mol. Biol. Reports 26, 95–101, 1999.

8. Rock KL, Gramm C, Rothstein L, et al. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class 1 molecules. Cell 1994;78:761–71.
9. Bailey JL, Wang X, England BK, Price SR, Ding X, Mitch WE. The acidosis of chronic renal failure activates muscle proteolysis in rats by augmenting transcription of genes encoding proteins of the ATP-dependent, ubiquitin-proteasome pathway. J. Clin. Invest. 1996;97:1447–53.
10. Medina R, Wing SS, Haas A, Goldberg AL. Activation of the ubiquitin-ATP-dependent proteolytic system in skeletal muscle during fasting and denervation atrophy. Biomed. Biochim. Acta. 1991;50:347–56.
11. Mitch WE, Medina R, Greiber S, et al. Metabolic acidosis stimulates muscle protein degradation by activating the ATP-dependent pathway involving ubiquitin and proteasomes. J. Clin. Invest. 1994;93:2127–33.
12. Price SR, Bailey JL, Wang X, et al. Muscle wasting in insulinopenic rats results from activation of the ATP-dependent, ubiquitin-proteasome pathway by a mechanism including gene transcription. J. Clin. Invest. 1996;98:1703–8.
13. Tawa NE, Odessey R, Goldberg AL. Inhibitors of the proteasome reduce the accelerated proteolysis in atrophying rat skeletal muscles. J. Clin. Invest. 1997;100:197–203.
14. Solomon V, Goldberg AL. Importance of the ATP-ubiquitin-proteasome pathway in degradation of soluble and myofibrillar proteins in rabbit muscle extracts. J. Biol. Chem. 1996;271:26690–7.
15. Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Ann. Rev. Biochem. 1996;65:801–47.
16. Qureshi AR, Alvestrand A, Danielsson A, et al. Factors predicting malnutrition in hemodialysis patients: A cross-sectional study. Kidney Int. 1998;53:773–82.
17. May RC, Bailey JL, Mitch WE, Masud T, England BK. Glucocorticoids and acidosis stimulate protein and amino acid catabolism in vivo. Kidney Int. 1996;49:679–83.
18. Price SR, England BK, Bailey JL, Van Vreede K, Mitch WE. Acidosis and glucocorticoids concomitantly increase ubiquitin and proteasome subunit mRNAs in rat muscle. Am. J. Physiol. 1994;267:C955–C960.
19. May RC, Kelly RA, Mitch WE. Mechanisms for defects in muscle protein metabolism in rats with chronic uremia: The influence of metabolic acidosis. J. Clin. Invest. 1987;79:1099–103.
20. Reaich D, Channon SM, Scrimgeour CM, Goodship THJ. Ammonium chloride-induced acidosis increases protein breakdown and amino acid oxidation in humans. Am. J. Physiol. 1992;263:E735–E739.
21. Reaich D, Channon SM, Scrimgeour CM, Daley SE, Wilkinson R, Goodship THJ. Correction of acidosis in humans with CRF decreases protein degradation and amino acid oxidation. Am. J. Physiol. 1993;265:E230–E235.
22. Graham KA, Reaich D, Channon SM, Downie S, Goodship THJ. Correction of acidosis in hemodialysis decreases whole-body protein degradation. J. Am. Soc. Nephrol. 1997;8:632–7.
23. Graham KA, Reaich D, Channon SM, et al. Correction of acidosis in CAPD decreases whole body protein degradation. Kidney Int. 1996;49:1396–400.
24. Lowrie EG, Lew NL. Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of the death rate differences among facilities. Am. J. Kid. Dis. 1990;15:458–82.

25. Movilli E, Zani R, Carli O, et al. Correction of metabolic acidosis increases serum albumin concentration and decreases kinetically evaluated protein intake in hemodialysis patients: A prospective study. Nephrol. Dial. Transpl. 1998;13:1719–22.
26. Stein A, Moorhouse J, Iles-Smith H, et al. Role of an improvement in acid-base status and nutrition in CAPD patients. Kidney Int. 1997;52:1089–95.
27. Mansoor O, Beaufrere Y, Boirie Y, et al. Increased mRNA levels for components of the lysosomal, Ca++-activated and ATP-ubiquitin-dependent proteolytic pathways in skeletal muscle from head trauma patients. Proc. Natl. Acad. Sci. USA 1996;93:2714–8.
28. Tiao G, Hobler S, Wang JJ, et al. Sepsis is associated with increased mRNAs of the ubiquitin-proteasome proteolytic pathway in human skeletal muscle. J. Clin. Invest. 1997;99:163–8.
29. England BK, Chastain J, Mitch WE. Extracellular acidification changes protein synthesis and degradation in BC3H-1 myocytes. Am. J. Physiol. 1994;260:C277–C282.
30. Isozaki Y, Mitch WE, England BK, Price SR. Interaction between glucocorticoids and acidification results in stimulation of proteolysis and mRNAs of proteins encoding the ubiquitin-proteasome pathway in BC3H-1 myocytes. Proc. Natl. Acad. Sci. USA 1996;93:1967–71.
31. Bailey JL, England BK, Long RC, Weisman J, Mitch WE. Experimental acidemia and muscle cell pH in chronic acidosis and renal failure. Am. J. Physiol. 1995;269:C706–C712.
32. Druml W, Kelly RA, May RC, Mitch WE. Abnormal cation transport in uremia: Mechanisms in adipocytes and skeletal muscle from uremic rats. J. Clin. Invest. 1988;81:1197–203.
33. Greiber S, O'Neill WC, Mitch WE. Impaired cation transport in thymocytes of rats with chronic uremia includes the Na+/H+ antiporter. J. Am. Soc. Nephrol. 1995;5:1689–96.
34. Greiber S, England BK, Price SR, Medford R, Ebb RG, Mitch WE. Na pump defects in chronic uremia cannot be attributed to changes in Na-K-ATPase mRNA or protein. Am. J. Physiol. 1994;266:F536–F542.
35. DeFronzo RA, Beckles AD. Glucose intolerance following chronic metabolic acidosis in man. Am. J. Physiol. 1979;236:E328–E334.
36. Bellocq A, Suberville S, Philippe C, et al. Low enviromental pH is responsible for the induction of nitric-oxide synthase in macrophages: Evidence for involvement of nuclear factor-kB activation. J. Biol. Chem. 1998;273:5086–92.
37. May RC, Kelly RA, Mitch WE. Metabolic acidosis stimulates protein degradation in rat muscle by a glucocorticoid-dependent mechanism. J. Clin. Invest. 1986;77:614–21.
38. Wing SS, Goldberg AL. Glucocorticoids activate the ATP-ubiquitin-dependent proteolytic system in skeletal muscle during fasting. Am. J. Physiol. 1993;264:E668–E676.
39. Tiao G, Fagan J, Roegner V, et al. Energy-ubiquitin-dependent muscle proteolysis during sepsis in rats is regulated by glucocorticoids. J. Clin. Invest. 1996;97:339–48.
40. Mitch WE, Bailey JL, Wang X, Jurkovitz C, Newby D, Price SR. Evaluation of signals activating ubiquitin-proteasome proteolysis in a model of muscle wasting. Amer. J. Physiol. 1999;276:C1132–C1138.
41. Lecker SH, Solomon V, Price SR, Kwon YT, Mitch WE, Goldberg AL. Ubiquitin conjugation by the N-end rule pathway and mRNA for its components increase in muscles of diabetic rats. J. Clin. Invest. (in press).
42. Chang HR, Bistrian B. The role of cytokines in the catabolic consequences of infec-

tion and injury. J. Parent. Ent. Nutr. 1998;22:156–66.
43. Herbelin A, Nguyen AT, Zingraff J, Urena P, Descamps-Latscha B. Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor a. Kidney Int. 1990;37:116–27.
44. Goodman MN. Tumor necrosis factor induces skeletal muscle protein breakdown in rats. Am. J. Physiol. 1991;260:E727–E730.
45. Flores EA, Bistrian BR, Pomposelli JJ, et al. Infusion of tumor necrosis factor/cachetin promotes muscle catabolism in rhe rat: A synergistic effect with interleukin 1. J. Clin. Invest. 1989;83:1614–1622.
46. Du J, Price SR, Mitch WE. Excessive muscle protein degradation in uremia: A novel mechanism for NFκB and glucocorticoids in the control of proteasome C3 subunit transcription. J. Amer. Soc. Nephrol. 1998;9:A3106.

DISCUSSION

DuBOSE, Houston: Bill, these are very interesting results and they are intriguing because of their simplicity as well in terms of the impact that simple bicarbonate administration might have on patients who have chronic renal failure. I want to ask you, now that you have extended activation of the ubiquitin proteosome pathway to other diseases that aren't always characterized by acidosis, can you tell us more about the afferent limb of this pathway? What is the signal? It is not always, I assume, pH or bicarbonate.

MITCH: Correct. We have a lot of complicated experiments to address your important question. These molecule-popping experiments strongly suggest that cytokines are heavily involved in activation of this system. Muscle proteolysis is not wholly due to activation of the ubiquitin proteosome system, there also is activation of proteases that are more proximal to the ubiquitin-proteosome system. These are activated first and then the resulting proteins can be "fed into" the system.

WINCHESTER, Washington: Bill, as you and I know, there are over 300,000 patients on dialysis in the United States alone, as of this year. The Holy Grail, of course, is something that will stop the progression of renal disease. Does bicarbonate have any effect on doing that?

MITCH: We don't have those data. There are some studies in rats in which bicarbonate was shown to slow down progression by interfering with complement activation. That story hasn't gone anywhere as far as I know, unless somebody else knows. It would be good if alkalinization did slow progression.

BRANSOME, Augusta: I would be very interested in your answer to the enthusiasm in some quarters, including FDA approval for an indication, for the administration of growth hormone in uremia. It seems as an antidote, if you will, to protein wasting. It seems to me that the two hypotheses are incompatible.

MITCH: Like everything in biology, things are often complicated. It turns out that growth hormone also suppresses protein breakdown, but the mechanism has not been well worked out. As to giving growth hormone to kidney patients, as far as I know there have been no long-term studies. There have been short-term studies and some of them have shown some benefit. Others are disappointing and most of the ones that I am familiar with have not been terribly well controlled. So I think these are things we have to work out because there really is a large problem here and we haven't solved it. It is so darn simple and cheap to give bicarbonate, that I would opt for that first.

HUMPHREYS, San Francisco: I want to follow up with the question that Tom addressed to you. There is a lot of interest now in the interaction between nutritional or

metabolic issues and inflammation in ESRD patients in promoting tissue catabolism. I wonder what your thoughts are about the nature of that interface and whether that is where the cytokine pathways you alluded to might be coming into play.

MITCH: Well, in fact, I perhaps made the acid story too simplistic, but it is appealing because we were able to manipulate it experimentally. It is certainly not the entire story. Our belief, based on experiments in cultured cells largely, is that it includes a problem with cytokines that are activating this system.

SMITH, Lawrence, Kansas: Bill, do your data allow a comparison in terms of protein breakdown between acute acidosis and chronic acidosis to which the body has adapted?

MITCH: The experiments that have addressed that have been in normal adults given acid loads. In those experiments, yes, indeed, acute metabolic acidosis will stimulate protein breakdown. The data I showed you we assume represent chronic acidosis; so probably both acute and chronic cases are comparable.

LUKE, Cincinnati: If 3000 nephrologists feed 300,000 dialysis patients with a lot of bicarbonate, some will get metabolic alkalosis. What does that do to protein metabolism?

MITCH: I should ask you as the expert on alkalosis to help me with that question. In the experiments that have been done in patients who are on dialysis, curiously, alkalosis has not produced the sort of problems that I would have worried about. It is remarkably safe. I talked with John Walls, who has had experience with inducing alkalosis in CAPD patients, and he told me that he has indeed found no real problems, such as hypertension, because of extra sodium.